AMIDE-BASED CATIONIC LIPIDS

This application is a continuation of 08/681,297, filed Jul. 22, 1996, now U.S. Pat. No. 6,020,526.

TECHNICAL FIELD

The present invention is directed to novel amide-based cationic lipid compounds useful in lipid aggregates for the delivery of macromolecules into cells.

BACKGROUND OF THE INVENTION

Lipid aggregates, such as liposomes, have been previously reported to be useful as agents for the delivery of macromolecules such as DNA, RNA, oligonucleotides, proteins, and pharmaceutical compounds into cells. In particular, lipid aggregates which include charged as well as uncharged lipids have been especially effective for delivering polyanionic molecules to cells. The reported effectiveness of cationic lipids may result from charge inmteractions with cells which are said to bear a net negative charge. It has also been postulated that the net positive charge on the cationic lipid aggregates may enable them to bind polyanions, such as nucleic acids. Lipid aggregates containing DNA have been reported to be effective agents for efficient transfection of cells.

The structure of various types of lipid aggregates vary depending on factors which include composition and methods of forming the aggregate. Lipid aggregates include, for example, liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, and may have particle sizes in the nanometer to micrometer range. Various methods of making lipid aggregates have been reported in the art. One type of lipid aggregate comprises phospholipid containing liposomes. An important drawback to the use of this type of aggregate as a cell delivery vehicle is that the liposome has a negative charge that reduces the efficiency of binding to a negatively charged cell surface. It has been reported that positively charged liposomes that are able to bind DNA may be formed by combining cationic lipid compounds with phospholipids. These liposomes may then be utilized to transfer DNA into target cells. (See, e.g. Felgner et al., *Proc. Nat. Acad. Sci.* 84:7413–7417, 1987; Eppstein et al. U.S. Pat. No. 4,897,355; Feigner et al. U.S. Pat. No. 5,264,618; and Gebeyehu et al. U.S. Pat. No. 5,334,761).

Known cationic lipids include N[1-(2,3-dioleoyloxy) propyl]-N,N,N-trimethyl-ammonium chloride ("DOTMA") and combinations of DOTMA with dioleoylphosphatidylethanolamine ("DOPE") are commercially available. Formulation of DOTMA, either by itself or in 1:1 combination with DOPE, into liposomes by conventional techniques has been reported. However, compositions comprising DOTMA have been reported to show some toxicity to cells.

Another commercially available cationic lipid, 1,2-bis (oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether, linkages to the propylamine. However, DOTAP is reported to be more readily degraded by target cells. Other cationic lipids which represent structural modifications of DOTMA and DOTAP have also been reported.

Other reported cationic lipid compounds include those which have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (See, e.g. Behr et al., U.S. Pat. No. 5,171,678).

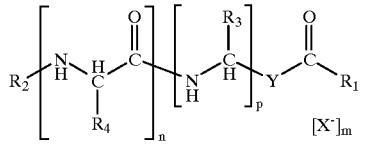
Another reported cationic lipid composition is a cationic cholesterol derivative ("DC-Chol") which has been formulated into liposomes in combination with DOPE. (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). For certain cell lines, these liposomes were said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions.

Lipopolylysine, made by conjugating polylysine to DOPE has been reported to be effective for transfection in the presence of serum. (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991).

However, of the cationic lipids which have been proposed for use in delivering macromolecules to cells, no particular cationic lipid has been reported to work well with a wide variety of cell types. Since cell types differ from one another in membrane composition, different cationic lipid compositions and different types of lipid aggregates may be effective for different cell types, either due to their ability to contact and fuse with target cell membranes directly or due to different interactions with intracellular membranes or the intracellular environment. For these and other reasons, design of effective cationic lipids has largely been empirical. In addition, to content and transfer, other factors believed important include, for example, ability to form lipid aggregates suited to the intended purpose, toxicity of the composition to the target cell, stability as a carrier for the macromolecule to be delivered, and function in an in vivo environment. Thus, there remains a need for improved cationic lipids which are capable of delivering macromolecules to a wide variety cell types with greater effeciency.

SUMMARY OF THE INVENTION

In one aspect of the present invention novel amide-based cationic lipids having the structure:



or a salt, or solvate, or enantiomers thereof are provided wherein; (a) Y is a direct link or an alkylene of 1 to about 20 carbon atoms; (b) R_1 is H or a lipophilic moiety; (c) R_2, R_3, and R_4 are positively charged moieties, or at least one but not all of R_2, R_3, or R_4 is a positively charged moiety and the remaining are independently selected from H, an alkyl moiety of 1 to about 6 carbon atoms or a heterocyclic moiety; (d) n and p are independently selected integers from 0 to 8, such that the sum of n and o is from 1 to 16; (e) X^- is an anion or polyanion and (f) m is an integer from 0 to a number equivalent to the positive charge(s) present on the lipid; provided that if Y is a direct link and the sum of n and p is 1 then one of either R_3 or R_4 must have an alkyl moiety of at least 10 carbon atoms.

In one embodiment R_1 may be a variety of lipophilic moieties including a straight chain alkyl moiety of 1 to about 24 carbon atoms, a straight chain alkenyl moiety of 2 to about 24 carbon atoms, a symmetrical branched alkyl or alkenyl moiety of about 10 to about 50 carbon atoms, a unsymmetrical branched alkyl or alkenyl moiety of about 10 to about 50 carbon atoms, a steroidyl moiety, a amine derivative, a glyceryl derivative, or $OCH(R_5R_6)$ or $N(R_5R_6)$, wherein R_5 and R6 are straight chain or branched alkyl moieties of about 10 to about 30 carbon.

In another embodiment when R_2, R_3, or R_4 are positively charged moieties it is preferable that the positively charged moiety be an alkylamine moiety, a fluoroalkylamine moiety, or a perfluoroalkylamine moiety of 1 to about 6 carbon